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Enterally Administered Antiserum Used in the Neutralization of Toxin
Formed in the Digestive Tract during Experimental Toxinfection with
Botulism Type A

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The reasons for conducting this research were data accumulated by us while studying the question of the toxinfectious nature in the pathogenesis of botulism. We determined that the pure toxin of the causative agents of botulism showed a considerably milder effect on the organism than the same dose of the toxin administered together with bacteria. The pure toxin, administered enterally, disappeared relatively fast from the digestive tract; even larger doses of the administered toxin could not be detected after 20 to 27 hours. Following a peroral administration, the toxin failed to appear in the blood after 2 to 3 days. In contrast with this, having administered enterally a culture of the causative agents of botulism, we regularly detected the toxin in the digestive tract (in the small intestine, particularly in its lower section and, according to the sequence of elimination, also in the stomach and in the large intestine), i.e. beginning with the 27th, 36th or 48th hour for 2 weeks

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and, sometimes, even after 20 to 30 days following the infection. We also detected the toxin in the blood of these animals for a long time afterward. The duration and the severity of the intoxication could be explained by additional production of the toxin by specific bacteria present in the organism.

After examination of the contents of the digestive tract in animals perorally infected with the culture of causative agents of botulism, the results proved that, the primary place where the production of the toxin takes place, is the small intestine.

Our data accumulated in the course of experiments substantiate the reported findings relevant to the presence of the toxin in the blood and in various organs, particularly in the intestines of cadavers of persons who died of botulism, often a long time after the ingestion of infected foodstuffs (PHIEDNAN and LORBER, SHAPIRO and NIKOLENKO).

The bibliographical data and own observations enabled us to establish our assumption that all cases of botulism in man, even those with a brief incubation period, should be treated as a toxin-infection, which, in its basicity, entrains additional intoxication in the organism by the toxin produced in the digestive tract. This circumstance is important not only from the theoretical standpoint, but much more so on account of its practical value. Here, the question arises whether the neutralization of the toxin, produced in the digestive tract, is advisable and feasible by applying a specific anti-toxin directly to the place of reproduction, in order to increase

the effectiveness of the serum therapy used in botulism.

In view of the discussed considerations, we decided that our objective will be a study of the effectiveness of the currently used intramuscular method of administration of the serum, also a combination of the intramuscular and enteral administration of the serum.

We performed 7 basic experiments on 57 rabbits, and 2 additional tests on 60 white mice and 20 guinea pigs. In each of the basic experiments we included three groups of animals with 2, 3 and 4 rabbits respectively. The animals in the first group were control animals and they were not subjected to the antiserum therapy; the animals in the second group received intramuscular injections of the serum; the animals in the third group received the serum by intramuscular and intraduodenal injections.

We developed in advance a special operative method of sewing the duodenal intestine to the incision in parietes of the peritoneum. Having made an incision in the peritoneal wall, we subsequently sutured the skin and muscles incompletely according to the size of the incision; consequently, a small opening remained in the wound, where we stitched the surface of the duodenal intestine at the bottom. We administered antiserum diluted in 20 to 25 ml of physiological solution of table salt directly into the duodenum with a hypodermic syringe by puncturing the duodenal wall with the needle. No postoperative complications were encountered, except in one case, which we shall discuss later. We believed that the endured

operation could have some effect on the condition of animals at the time of the toxin infection's reproduction, consequently we performed simultaneously a control operation on animals of two groups, namely an incision through all layers of the abdominal wall and then we sutured the wound.

Several days after the described operative preparation, we infected the animals perorally with a whole 9-day culture of *Clostridium botulinum* type A-98 (the cultivation was performed in MARTIN'S broth with glucose and liver under anaerobic conditions). We administered the culture in various tests in doses of 1.5 to 2.5 Dlm.

The experimental results were evaluated by the phagocytic index method according to the manifested symptoms and the mortality of animals, as well as according to the availability of the toxin in the blood and in various sections of the digestive tract. Prior to this, while studying the pathogenesis of botulism, we obtained data, which verified the sensitivity of this method as being considerably greater, than that of the classic biological test, and also we found some relevant indications in the literature (KOVTONOVICH, MINERVIN et al., SAVIN, ESSEL).

The basic experiments varied only by the quantities and frequency of the administered toxin.

The experiments No.1 and 2 were performed on 12 rabbits. They became infected with the 1.5 Dlm dose of the culture administered to each animal. Then, they received a single injection of the serum 14 hours after the infection. During the therapy by the intramuscular method, we administered the serum in volumes of 1.000 BU;

we included in the combination method $\frac{1}{3}$ of the injection volume by the intramuscular application and the $\frac{2}{3}$ volume by the intraduodenal administration. As a result, 2 out of 4 control rabbits died after intramuscular treatment, one of them died 7 days after the infection; out of 4 rabbits, which received a treatment by the combination method, one animal died 15 days after the infection. The results of the latter experiment, if only the survival time of the animal should be judged, did not permit us to draw any conclusion as to the greater effectiveness of the combination method of the serum administration, than by the intramuscular method. A comparative study of the dynamics of intoxication in an organism produced diverse results. We established that, 10 hours after intramuscular administration of the serum (24 hours after the infection), the toxin was detected in the blood of two animals and, in the other two animals, the toxin was not found in the blood, i.e. the toxin was neutralized. As a rule, beginning with 48 hours after the infection, we detected, during 7 to 8 days, the presence of the toxin in the blood of all 4 animals which received intramuscular treatment, and we also discovered the toxin in the digestive tract of one dead rabbit. This could be interpreted that, the recorded by us existence of the toxin in the blood, regardless of the intramuscular administration of a larger serum dose for one rabbit (1,000 IU), was the result of the admittance of newly supplied quantities of the toxin to the blood stream from the digestive tract. At the same time, following the treatment with serum by the combination method, the toxin disappeared from the blood stream in all 4 animals. Moreover,

the toxin could not be detected in two other rabbits; we recorded a brief appearance of the toxin in the blood stream of one animal on the 10th and 12th day after the infection. And, in the other rabbit of this group, which died on the 15th day, the toxin appeared in the blood on the 5th day, and continued to reappear until death; we also detected the toxin in the digestive tract of the same animal.

If we take into account the fact that, with the combination treatment, the animals received at that time only 330 BU of the serum, while with the intramuscular treatment - full 1,000 BU, we think the obtained results proved that the intoxication of a lesser intensity existed in animals treated by the combination method as a result of the intraduodenal administration of the serum. Apparently, the additional intoxication of the organism by the toxin absorbed from the digestive tract was either discontinued, or reduced.

Yet, the circumstance that in this experiment on two rabbits all symptoms of the specific toxinfection developed subsequently, induced us to draw a conclusion that it is necessary to administer a larger dose of serum in order to increase its therapeutic effect.

The experiment No.3 was conducted on 6 rabbits. We infected the animals with 2 Dlm of the culture. Two untreated control rabbits died after 36 hours following the infection, and the botulinal toxin was detected in their blood and in the digestive tract. Only 3 rabbits were exposed to the serum treatment, because one rabbit died from septicopyemia, which developed as a result of the operation. We detected botulinal toxin in the blood and in the digestive tract

of this rabbit, and this indicated that the toxin infectious process had already developed. Out of 3 remaining rabbits, two received intramuscularly 2,000 BU of the serum each, 2 1/2 days after the infection. As a result, a complete neutralization of the toxin took place in the blood of one animal, then, in the blood of another rabbit, a neutralization of the toxin was noted over a period of 8 days, and, subsequently, the toxin reappeared in the blood of the same rabbit, remaining there until death of the animal on the 12th day. We also detected the toxin in the digestive tract of the discussed animal after death. Two and a half days after the infection, we administered intramuscularly 1,000 BU of the serum to one rabbit, which was prepared for the combination method of treatment, and we also applied the same quantity of the serum by duodenal injection. The rabbit was in a very grave condition at the time of the injection of serum, and died in 20 hours after the injection. Yet, we failed to detect the toxin in the blood and in the digestive tract of this animal; this indicated that the neutralization of the toxin in the intestine occurred after the administration of serum (the toxin was detected in the digestive tract of untreated animals). This animal's death can be explained so that, at the time of the administration of serum (2 1/2 days after the infection), the absorbed toxin has already made an irreversibly fatal effect on the organism. This experiment is of no value from the standpoint of a comparative study of the effectiveness of the serum administration methods. However, a consideration should be given to the established fact that the toxin can be neutralized in the digestive tract after intra-

duodenal administration of the serum.

In the course of the next 4 experiments we administered 500 BU of the serum to all rabbits intramuscularly and also 10,000 BU of the serum by duodenal application to each animal, according to the combination method. In the intramuscular and by the combination method of treatment, we administered the serum two times: in one experiment, after 14 and 36 hours, and in the second experiment, after 36 and 48 hours; then, in the remaining two instances we administered the serum after 24 and 48 hours, in each case following the time of infection. Subsequently, the animals exposed to the combination treatment method received only the intraduodenal injection and, during this time, we administered in various experiments from 3 to 5 additional intraduodenal injections, with intervals of 1 or 2 days. In these experiments we used infectious doses equal from 2 to 2.5 Dlm of the culture.

Out of 13 untreated control rabbits, 11 died in these experiments in the first 2 or 3 days after the infection. A developed toxinfection was proven after detection of the toxin in the small intestine. Out of 13 rabbits which received the intramuscular treatment, 8 died. After administration of the serum the neutralization of the toxin in the blood failed completely in 3 rabbits and they died after 5 to 7 days following the infection. In the remaining 10 animals, we observed the neutralization of the toxin in the blood after intramuscular administration of the serum, and this usually occurred after the second injection. In 8 out of these animals, the toxin in the blood reappeared several days later and

5 rabbits died as a result of the developed toxin infection (the toxin was detected in the intestines), but 3 rabbits recovered. We subsequently observed, in two rabbits only, a complete neutralization of the toxin in the blood and the absence of additional intoxication. Thus, out of 13 rabbits which received the intramuscular treatment, we found only 2 in which a good therapeutic effect was manifested, while 11 animals developed a toxin infection. The additional intoxication of the organism in 11 rabbits was of a transitory type in 3 animals only; the latter, subsequently, recovered, while 8 rabbits died as a result of the developed toxin infection. In contrast with the above findings, out of 13 rabbits treated by the combination method, none of the animals died; the rabbits endured their sickness better and they recovered sooner than the animals treated by the intramuscular method. Shortly after the administration of the serum, and during the entire course of the experiment, no toxin was found in their blood. The toxin in the blood appeared only in one rabbit; its presence was recorded between 6 and 8 days after the infection, which corresponded in time with a previous 2-day interval in the intraduodenal administration of the serum (5th and 6th day).

Thus, out of 19 untreated control animals, 15 died; out of 19 animals which received the intramuscular treatment, 10 died; but only 2 animals died out of 18, which received a combination serum treatment. During the intramuscular treatment, the process of additional intoxication of the organism by the toxin was not discontinued and the toxin was still produced in the ~~digestive~~ tract.

The intramuscular administration of the serum, when supplemented with the intraduodenal injection, proved to be considerably more effective under our experimental conditions than the intramuscular treatment alone. Thus, it can be assumed that the serum administered by the duodenal method neutralizes the toxin produced in the small intestine.

In the described by us experiments on rabbits, we hardly had a chance to observe directly in the small intestine the neutralization of the toxin, because, usually, all animals survived. In additional experiments on infected white mice and guinea pigs, we administered antitoxic serum diluted with a 0.5% solution of soda directly into the stomach by means of a catheter to demonstrate a possibility of bringing the serum to the lower section of the small intestine in order to neutralize the botulinal toxin that forms there. After administration of the serum, we failed to detect the toxin in the small intestine of these animals; meanwhile, we usually detected the toxin in the small intestine of untreated control animals.

We obtained data relevant to the destination of the enterally administered serum and they indicated that: after the antitoxic serum had been admitted to the contents of the small intestine in animals infected with the botulinal toxin, it disappeared considerably slower than when administered to healthy animals. Apparently, this circumstance can be explained by a decreased activity of the digestive enzymes in animals poisoned with the toxin.

As a result of the research which involved the nature and dynamics of the developed intoxication, and also pertinently to the

place where the basic production of new quantities of the toxin takes place in the organism, we established experimentally the combination method (intramuscular - enteral), which can be used in the treatment of botulism with the injected serum. This means in a practical application that, for the treatment of natural botulism in man, one needs a 50,000,000 BU volume of the serum diluted with boiled water to 300 or 400 ml, which should be administered by means of duodenal catheter. We can also recommend the administration of serum directly into the rectum by the deep-clysm method.

The priority in suggesting the enteral method of administration of serum in cases of botulism belongs to S.V. WINERVIN, who, on the basis of theoretical premises, used this method in 1948 on 5 patients stricken with botulism and obtained favorable results.

Under our observation was one patient who suffered from botulism type B, which run a mild course. Then, having performed the opsonin-phagocytic test on the patient, we detected the toxin in his blood in spite of the intramuscularly applied three hypospecific injections of serum of 50,000 BU each. Finally, the toxin disappeared from the blood after a single duodenal injection of 50,000 BU of the serum.

We believe that, our method of treatment of botulism having been experimentally tested and producing favorable results in cases of botulinal toxin infection we developed artificially in animals, deserves a consideration and it should be used in cases of botulism

involving human patients.

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